Claims

1. A method for the individual adaptation of excitation intensities in a multiband fluorescence microscope having several spectrally different excitation bands that are simultaneously converted from an fluorescence object (12) into fluorescence bands having fluorescence intensities,
wherein
a) the fluorescence intensities of the individual fluorescence bands in the microscopic image are determined and compared to intensity setpoint values larger than or equal to zero,
b) for each excitation band that is assigned to a fluorescence intensity deviating from the intensity setpoint values, a selective filter (23; 28, 29, 31, 32) is brought into the illumination beam path.
c) and the transmission factors of the individual filters (23; 28, 29, 31, 32) in effect in the illumination beam path are continuously adjusted so that by attenuating the associated excitation bands, all fluorescence intensities are adjusted to their intensity setpoint values.

2. The method as recited in Claim 1,
wherein
the setpoint values for the different fluorescence intensities are all equal to the lowest fluorescence intensity.

3. The method as recited in Claim 1,
wherein
at least one of the setpoint values for the different fluorescence intensities is equal to zero.

4. The method as recited in Claim 1,
wherein
for some fluorescence intensities the setpoint values are equal to zero and for the rest are equal to the lowest fluorescence intensities.

5. The method as recited in Claim 1,
wherein
the different fluorescence intensities are visually determined.

6. The method as recited in Claim 1,
wherein
the different fluorescence intensities are determined using an intensity meter.

7. The method as recited in Claim 6,
wherein
the different fluorescent intensities are determined using a CCD or a video camera (18) having an image analysis system (19).

8. The method as recited in Claim 1,

wherein

a) modifications of the fluorescence intensities are continuously determined,

b) and by repeated adaptation of the transmission factors of the filters (23; 28, 29, 31, 32) in effect in the illumination beam path, the fluorescence intensities are always brought back to their setpoint values.

9. The method as recited in Claim 2,

wherein

a) modifications of the fluorescence intensities are automatically continuously determined,

b) and by automatic continuous adaptation of the transmission factors of the filters (23; 28, 29, 31, 32) in effect in the illumination beam path, the fluorescence intensities are always kept at their setpoint values.

10. A multiband fluorescence microscope for utilizing the method as recited in Claim 1, including an illumination beam path having a light source (1), a collector (3), a multiplicity of lens members (4, 6, 8), an aperture diaphragm (5), a radiant field diaphragm (7), an excitation filter (9) for simultaneous production of several excitation bands of different light wavelengths and a filter element to affect these excitation bands, also including a beam splitter (10) and an objective (11) that directs the illumination beam path onto a fluorescence object (12) on a specimen stage (13) and projects it through the beam splitter (10), an output filter (14) and a tube lens (15) into an intermediate image plane (16),

wherein

a) a filter draw assembly (20) made of individually movable, tightly spaced filter draws (21; 21a, 21b) is inserted perpendicular in the illumination beam path tightly next to the aperture diaphragm (5),

b) a selective filter (23; 28, 29, 31, 32) is provided on each filter draw (21) for each excitation band that has surface regions with high and low transmission factors,

c) the surface region having the lowest transmission factor has a minimum diameter equal to the beam diameter (x) to completely cancel the excitation band,

d) a blank aperture (22) having the beam diameter (x) is arranged next to each filter (23; 28, 29, 31, 32),

e) the transmission factor of each filter (23; 28, 29, 31, 32) diminishes as one moves further away from the blank aperture (22),

f) and separate positioning means (25), with which any surface region of the filter draw (21) can be inserted in the illumination beam path, are assigned to each filter draw (21).

11. The fluorescence microscope as recited in Claim 10,
wherein
to affect a number n of excitation bands

a) the filter draw assembly (20) consists of n-1 filter draws (21) on separate, tightly spaced n-1 layers/planes parallel to the aperture diaphragm plane (24),

b) each filter draw (21) has at least one blank aperture (22) and n selective filters (23; 28, 29, 31, 32) for the n excitation bands.

12. The fluorescence microscope as recited in Claim 10,
wherein
to affect two excitation bands A, D a single filter draw (21) is provided,

a) that has on its one end a long-pass filter (29) to weaken the intensity of the short-wave excitation band A,

b) that has on its other end a short-pass filter (28) to weaken the intensity of the long-wave excitation band D,

c) and has the blank aperture (22) in between them,

d) and positioning means (25) for continually shifting the filter draw (21) are mounted parallel to the aperture diaphragm plane (24).

13. The fluorescence microscope as recited in Claim 11,
wherein
a) the filter draw (21) has a transparent, rectangular glass plate (27) on whose ends the short-pass filter (28) and the long-pass filter (29) applied as vapor deposition layers are opposite one another,
b) and the short-pass filter (28) and the long-pass filter (29) each have an increasing portion of the vapor-deposited glass surface, and as a result a decreasing transmission as one moves from the blank aperture (22) toward the ends of the filter draw (21).

14. The fluorescence microscope as recited in Claim 12,
wherein
the areas of the two vapor-deposition layers at the ends of the filter draw (21) each have the form of a rectangle having a minimum edge length equal to the beam diameter (x) against which the base of a vapor-deposited, isosceles triangle area borders in the direction of the blank aperture (22).

15. The fluorescence microscope as recited in Claim 12,
wherein
the vapor-deposition layers are applied neither wholly or in part as connected are, but rather as area elements (30) whose size or distances are selected differently as one moves in the direction of shifting from the blank aperture (22) toward the ends.

16. The fluorescence microscope as recited in Claim 10,
wherein
a two-piece filter draw assembly (20) is provided to affect three excitation bands A, B, D,

a) each filter draw (21) having a circular blank aperture (22) in the center,

b) three selective filters (28, 29, 31) for the excitation bands A, B, D having a transmission factor that diminishes as the radius increases are arranged as sectors around the center,

c) and separate positioning means (25), with which the filter draws (21) can be shifted independently of each other parallel to the aperture diaphragm plane (24) and or rotated, are assigned to each filter draw (21).

17. The fluorescence microscope as recited in Claim 16,
wherein
to achieve a transmission factor that diminishes as the radius increases, the portion of the vapor-deposited area of the filter (28, 29, 31) increases as the radius increases.

18. The fluorescence microscope as recited in Claim 10,

wherein

a two-piece filter draw assembly (20) made of two circular filter draws (21) is provided to affect three excitation bands A, B, D,

a) each filter draw (21) being divided into six sectors covering the illumination beam path and being arranged around its center situated outside the beam path,

b) every second sector being a blank aperture (22) and with one of the three selective filters (28, 29, 31) for the excitation bands A, B, D being situated between each pair.

c) each filter (28, 29, 31) exhibiting an increase of the transmission factor in one of the two directions of rotation.

d) and separate positioning means (25) being provided for rotating each filter draw (21) around its center and parallel to the aperture diaphragm plane (24).

19. The fluorescence microscope as recited in Claim 10,

wherein

a two-piece filter draw assembly (20) made of two rectangular filter draws (21) is provided to affect three excitation bands A, B, D,

a) each filter draw (21) having a blank aperture (22) in the center and having at both of its ends two different combinations of two out of three of the selective filters (28, 29, 31) for the excitation bands,

b) the transmission factor of the filters (28, 29, 31) diminishing in the longitudinal direction of the filter draw (21) moving from the blank aperture (22) toward the ends,

c) and separate positioning means (25) being arranged for shifting the filter draw (21) in the longitudinal direction and parallel to the aperture diaphragm plane (24).

20. The fluorescence microscope as recited in Claim 10,

wherein

a three-piece filter draw assembly (20) made of three circular filter draws (21) is provided to affect four excitation bands A, B, C, D,

a) each filter draw (21) having a blank aperture (22) in the center and four selective filters (28, 29, 31, 32) situated around said center for the excitation bands as vapor-deposited ring sectors that border each other and have a transmission factor that decreases as the radius increases,

b) and separate positioning means (25) being provided parallel to the aperture diaphragm plane for shifting and/or rotating each filter draw (21).

21. The fluorescence microscope as recited in Claim 10, wherein

a three-piece filter draw assembly (20) made of three circular filter draws (21) is provided to affect four excitation bands A, B, C, D,

a) each filter draw (21) being divided into eight sectors covering the illumination beam path and being located pivoted around its center, which lies outside the beam path,

b) every second sector being a blank aperture (22) and in between them being situated one of the four selective filters (28, 29, 31, 32) for the excitation bands,

c) each filter (28, 29, 31, 32) showing an increase in the transmission factor in one of the two directions of rotation,

d) and separate positioning means (25) being provided for rotating each filter draw (21) around its center and parallel to the aperture diaphragm plane (24).